



# Ant (*Formica aerata*) Predation on Larvae of *Diorhabda elongata* (Coleoptera: Chrysomelidae), a Biological Control Agent Introduced Against Saltcedar (*Tamarix ramosissima*) in the Western U.S.

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## Abstract

Anti-exclusion experiments were conducted to quantify the survivorship of *Diorhabda elongata* in the field. Cohorts of first instar larvae were placed on saltcedar (*Tamarix ramosissima*) branches that were either exposed or protected from ants. The branches were located in areas with either "high" or "low" ant activity. The mean daily survival rates for larvae protected from ants in both high (rate=0.86) and low (rate=0.85) ant activity areas were significantly higher when compared to larvae exposed to ants. Larvae exposed to ants had a mean survival rate of 0.40 and 0.72 when located in areas of high and low ant activity, respectively.

## Introduction

Saltcedar, *Tamarix ramosissima*, is an invasive plant and a target for an interagency biological control program. Saltcedar is a major problem in the western United States because it has replaced native vegetation in approximately 500,000 hectares of riparian habitat<sup>1</sup>. Some of the ecological impacts due to saltcedar invasions are degradation of riparian wildlife habitat and reduction in abundance and diversity of native wildlife<sup>2</sup>.

The leaf beetle, *Diorhabda elongata*, is native to Eurasia from which a Chinese strain was selected and recently introduced in the US to help control saltcedar. The beetle was released in multiple sites in six western states, including the Owens Valley of California. The importation and release of an insect to a new environment offers a unique opportunity to conduct post-release survivorship studies. Introduced insects have traditionally met with biotic resistance to establishment in the form of predation, parasitism and/or disease in their new environments<sup>3</sup>.

After *D. elongata* was released, observational studies were conducted to identify the sources of mortality that the beetle encountered. These observations indicated that ants (*Formica aerata*) were a major predator of *D. elongata* larvae. Ants would either carry away the larvae or cause the larvae to drop from a branch to escape capture, leaving the larvae vulnerable to ants or other predators. Based on these findings, the present ant-exclusion study was conducted. The objective of this study was to evaluate the impact of ant predation on the survivorship of *D. elongata* larvae in the field.



*D. elongata* 3<sup>rd</sup> instar larva



*Formica aerata*

## Materials and Methods

- Saltcedar trees were selected in our Owens Valley research site in areas of both "high" and "low" ant activity. High and low ant activity was determined by making visual assessments of the number of ant visitations per unit length of branch.
- One branch per saltcedar tree, approximately one meter from the ground, was assigned to one of two treatments: (1) **Exclusion of ant predation**: a ring of Tanglefoot® was applied to the bottom of the branch and adjacent vegetation was cleared so that ants could not climb onto the branch, and (2) **Control** (ants not excluded). The study was replicated 11 times over one a month period.
- Forty one day old 1<sup>st</sup> instar larvae (laboratory reared) were placed on each branch and monitored daily until they were killed or pupated.

Analysis of data:  
• Estimating the mean daily survival rate from survivorship curves: The daily survival rate was estimated for the period of time between the first day of the experiment and a final cut-off day. The cut-off day corresponds to the day we counted the maximum mean number of 3<sup>rd</sup> instar larvae. The mean number of 3<sup>rd</sup> instar larvae starts to decrease after the cut-off day, after which we cannot separate disappearance between mortality and pupation. A t-test was used to compare mean daily survival rates between the two treatments.

- **Low ant activity area:** All three larval stages developed and survived in both ant exclusion treatments. The 1<sup>st</sup> and 2<sup>nd</sup> instar percent survival estimates from both treatments were also similar. When ants were excluded, 29% of the 1<sup>st</sup> instar and 88% of the 2<sup>nd</sup> instar larvae survived (Fig. 3A). When ants were present, the 1<sup>st</sup> and 2<sup>nd</sup> instar survival rates were 21% and 88%, respectively (Fig. 3B).

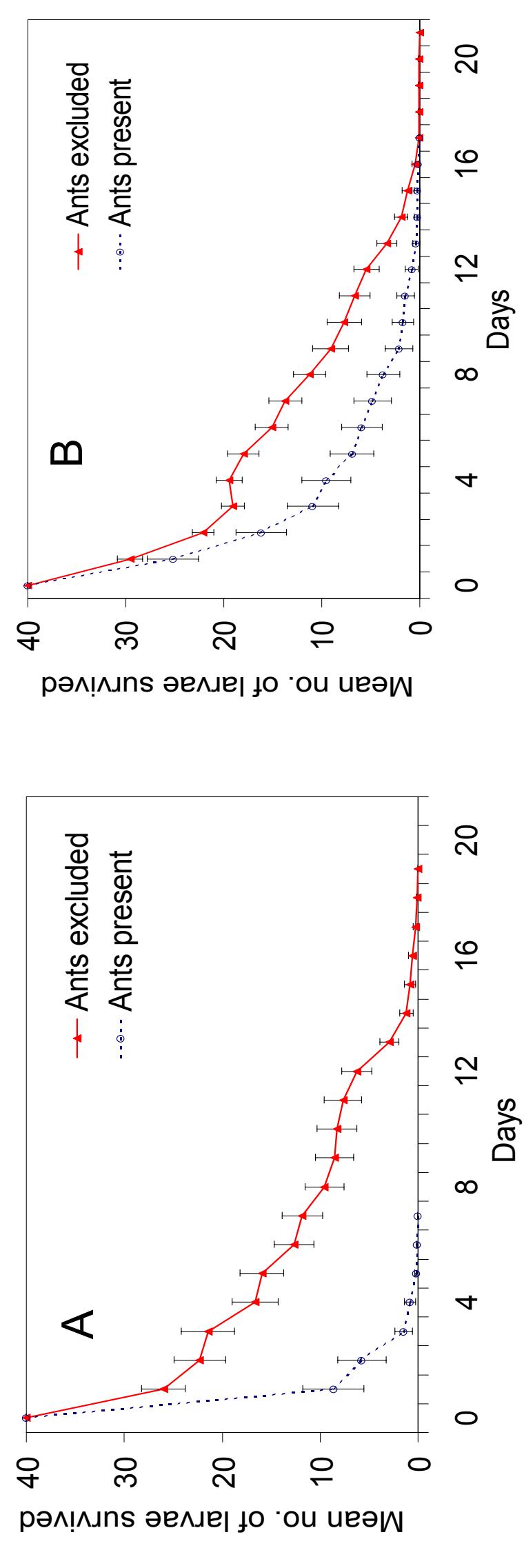
**Figure 3.** Stage-specific survivorship curves for larvae located in areas of low ant activity. Treatments: A) ants excluded and B) ants present.

## Results and Discussion

Mean daily survival rate:  
• **High ant activity area:** The mean daily survival rate of larvae from the two ant exclusion treatments were statistically different ( $t=-6.581$ ;  $df=15$ ;  $P=0.0001$ ). The mean daily survival rate for larvae was  $0.86 \pm 0.04$  when ants were excluded and  $0.40 \pm 0.05$  when ants were not excluded. The survivorship curves (Fig. 1A) demonstrated differences in survival. In addition, there was a significant difference in larval longevity between the two treatments; larvae lived a maximum of 7 days when exposed to ants, compared to a maximum of 20 days when ants were excluded. Many of the individuals protected from ants pupated while few in the high ant treatment survived the larval stage.

• **Low ant activity area:** Similar to high ant activity areas, the difference between the mean daily survival rate between the two treatments was statistically significant ( $t=-2.780$ ;  $df=17$ ;  $P=0.0128$ ). Larvae attained a  $0.85 \pm 0.03$  survival rate on branches protected from ants; whereas larvae on branches exposed to ants had a survival rate of  $0.72 \pm 0.65$  (Fig. 1B).

**Figure 1.** Cumulative survivorship curves for larvae located in areas of A) high and B) low ant activity.



## Stage-specific survival:

- **High ant activity area:** All three larval instars of *D. elongata* developed and survived to the experimental cut-off date when ants were excluded, however, survival rates for each larval stage were different. The estimated, average percent survival for the 1<sup>st</sup> and 2<sup>nd</sup> instar was 30% and 70% respectively (Fig. 2A). In contrast, when ants were not excluded, only 5% of 1<sup>st</sup> instar larvae survived to the 2<sup>nd</sup> instar, but none of these 2<sup>nd</sup> instars survived to the 3<sup>rd</sup> instar (Fig. 2B).
- Low ant activity areas: larvae survived to the third instar regardless of ant exclusion treatment, however ants still produced significant levels of mortality that may limit the effectiveness of *D. elongata* in some areas.
- This was the first study conducted to quantify the effect of ant predation on *D. elongata* survivorship in the field. We plan to expand our work in this area of research in the future, so that we can improve management strategies for saltcedar biological control programs.

**Figure 2.** Stage-specific survivorship curves for larvae located in areas of high ant activity. Treatments: A) ants excluded and B) ants present.

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